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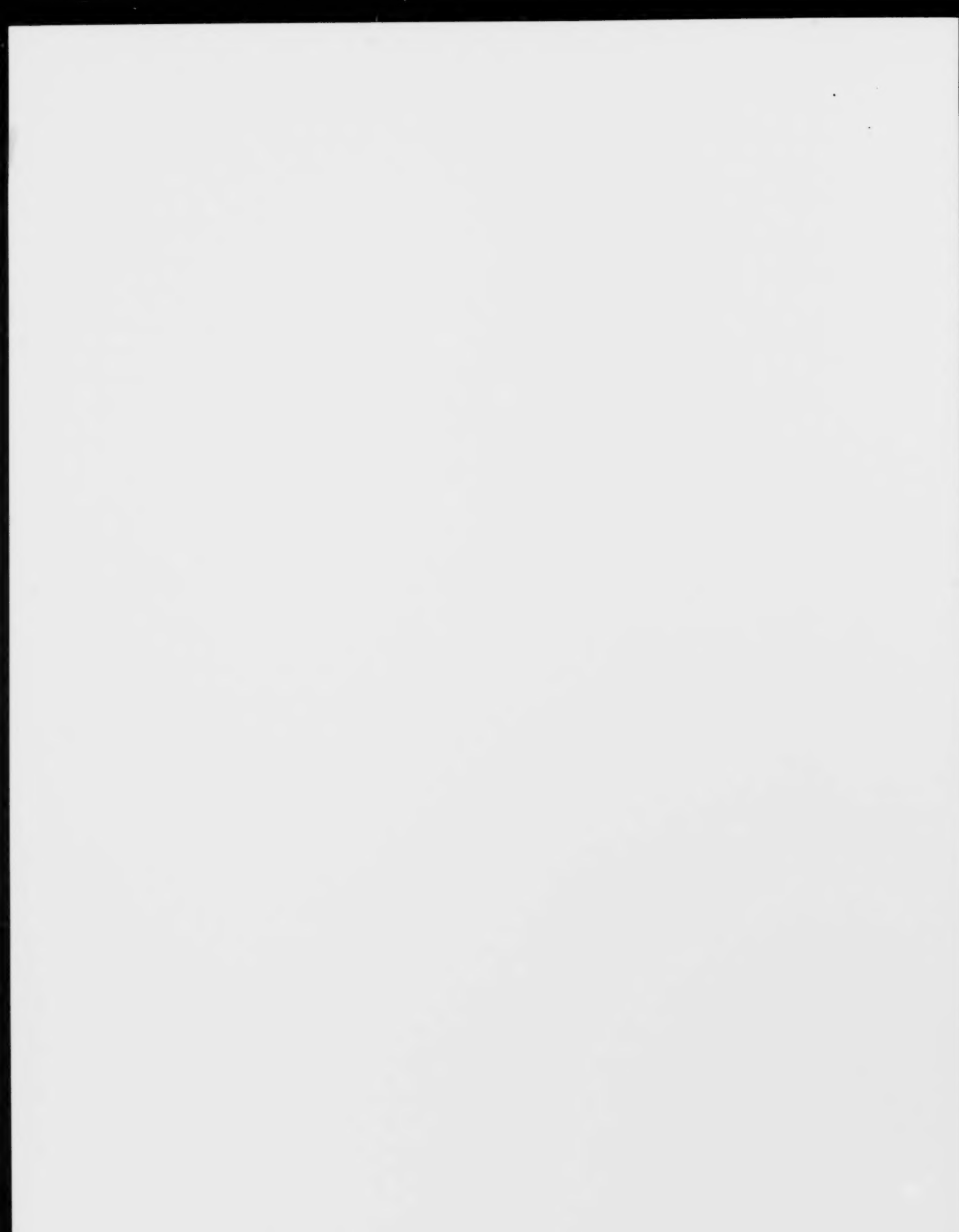
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**PORCINE ADENOVIRUS-3 BASED VACCINE FOR PORCINE
RESPIRATORY AND REPRODUCTIVE SYNDROME VIRUS**

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FINAL REPORT

Saskatchewan Agriculture Development Fund

PROJECT # 20030551

**"Porcine adenovirus-3 based vaccine for porcine respiratory
and reproductive syndrome virus"**

By

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a) Abstract/Summary

Vaccination against infectious diseases is considered to be one of the major advances in modern medicine when one considers the reduction in human and animal suffering. Sub-unit vaccines often do not induce breadth of immunity required (e.g. cell mediated immunity etc.), and they require strong adjuvants, of which few are licensed. Furthermore, producing sub unit vaccines for use in veterinary medicine, using modern biotechnology approaches is dictated by economics. Thus, novel approaches are required to produce animal vaccines to reduce animal suffering and productivity. One feasible way of achieving this is to develop safe and effective and economically viable live-vectored vaccines. The main objective of this grant was to develop porcine adenovirus -3 (PAdV-3) based porcine respiratory and reproductive syndrome (PRRS) vaccine by improving the expression of vaccine antigens. The PAdV-3 infects pigs but often does not produce disease. Earlier, we characterized PAV-3 at the molecular level by determining the complete genome sequence and transcriptional map.

We have demonstrated the feasibility of constructing synthetic genes and isolating recombinants PAdV-3s expressing these genes of PRRS virus. Our preliminary results suggest that optimising the codon bias of genes has helped to increase the expression of PRRS virus genes. This increased expression led to enhanced immune response in challenged pigs. However, our preliminary results suggest that immunization of pigs with recombinant PAdV-3 expressing PRRS antigens did not protect animals in protection / challenge experiments. Further experiments need to be done before full potential of this approach (PAdV-3 vector) can be realized.

b) Introduction

The use of vaccines is an effective method of stimulating the naïve animal to develop protective immunity against the disease causing organism. Since many disease causing organisms enter at mucosal surfaces (respiratory and gastrointestinal tract), immunity is required at the mucosal site to block the initial infection and thus reduce the chances of development of disease. As compared to sub-unit vaccines, live vaccines are best inducers of mucosal immunity when administered orally or intranasally. In addition, production of live vaccines is cost effective. However, use of live vaccines produced by conventional means ensures that the live organism is always present in the animals which many times can mutate back to virulent form (in vivo recombination) and cause fatal disease. Thus, new approaches have to be developed for the safe and cost effective production of viral vaccine antigen. One way to achieve this is to develop live vectored vaccines. Such live vectors can be engineered to carry genes for other pathogens thus making it possible to immunize animals to produce protective immunity at the mucosal surface to various disease organisms at one time. This will not only improve vaccination, but will also reduce the cost of producing vaccines.

We have chosen PAdV-3 for the development of such vaccines as it infects porcine respiratory and gastrointestinal tract and induces mild disease^{1,2}. By genetically engineering this virus, we can cripple (E3 deletion) it so that it will not cause disease. It can be further crippled by an E1 deletion so that it can infect and produce the proteins but not assemble into the virus particles. Introduction of foreign genes (respiratory / enteric viruses of porcine) into different regions of PAdV-3 genome, will not only reduce the pathogenicity of the virus, but will also make PAdV-3 as a safe and effective vehicle for

delivering vaccine antigens at the mucosal surfaces. We have chosen porcine respiratory and reproductive syndrome disease of pigs as a model since it causes significant economic losses due to pneumonia in young pigs, reproductive failures in sows and an increase in preweaning mortality³. Although the successful control of the disease depends on vaccination, however the *efficacy of the currently available vaccines has been a major issue*. Some of the recent outbreaks of PRRS disease have been linked to the use of live attenuated vaccines. Moreover, use of current vaccines does not allow differentiation between vaccinated and infected animals. *Use of PAdV-3 based PRRS vaccine a) will eliminate the problems associated with the use of live attenuated PRRS vaccines and b) will allow to differentiate between vaccinated and infected pigs.*

The main objective is to develop a generic delivery system for viral antigens for use in porcine as effective vaccine using PRRS as a model.

The specific aims are:

- a) Construction of synthetic PRRS virus genes (Orf5 and Orf3), and confirm the identity of synthetic genes
- b) Construction of recombinant PAdV-3 expressing a) single antigen b) double antigen
- c) Testing immunogenicity and protection in pigs

c) Methods:

Earlier we identified the regions (e.g. E1, E3 and E4), which can be used for the insertion of foreign genes^{4,5,6}. In addition, we have also developed an easy method of constructing recombinant PAdV-3. Using homologous recombination machinery of

E.coli, we constructed a full length PAdV-3 genomic clone in a plasmid⁷. This plasmid DNA when transfected into porcine cells produced virus, which was identical to wild-type PAdV-3. This will be used to introduce deletions/foreign genes in PAdV-3 genome in a plasmid and then using plasmid DNA for isolating recombinant PAdV-3.

a. Construction of synthetic PRRS genes.

In order to obtain optimal expression of PRRS genes, Orf5 and Orf3 genes will be reconstructed in vitro by adjusting the codon bias (based on usage of codons in abundantly expressed proteins of pig) and by removing cryptic splice site sequences of these genes without affecting the amino acid sequence of Orf5 and Orf3. This will be achieved by synthesizing and ligating oligonucleotides of desired DNA sequence. The identity of the resynthesized genes will be confirmed by determining the DNA sequence.

b. Construction of recombinant PAdV-3 expressing single gene.

In order to construct recombinant PAdV-3 expressing PRRS gene, the coding sequence of synthetic Orf 5 or Orf 3 gene will be inserted in a E3 transfer plasmid and recombined into E3/E4 deleted full length PAdV-3 genomic clone using homologous recombination machinery of *E. coli*. The porcine cells will be transfected with individual plasmid DNA to isolate recombinant virus. The identity of the virus will be verified by restriction enzyme analysis of virion DNA and by Western blot/immunoprecipitation of Orf5 or Orf3 protein from recombinant virus infected cells.

c. Construction of recombinant PAdV-3 expressing multiple genes.

Initially, we will use the strategy of fusing the coding sequences of two genes in such a way that the coding sequences are separated by a 500 bp internal ribosomal entry site (IRES) fragment from endomyocarditis (EMC) virus. This will produce a single mRNA in which the 5' gene will be translated by a ribosomal scanning method while the 3' gene will be translated by an internal ribosomal entry site mechanism (afforded by EMC sequence). In this instance, initially the 5' untranslated region of endomyocarditis (EMC) virus will be inserted between the genes coding for Orf5 and Orf3. The whole transcriptional unit will be inserted in E3 transfer plasmid before recombining into E3 deleted full length PAdV-3 genome. Second strategy will be to insert two genes at different insertion sites, In this instance, Orf5 gene will be inserted in E3 region and Orf3 gene (under appropriate transcriptional elements) will be inserted in E4 region of E3-E4 deleted PAdV-3 genome. Once full length plasmid DNAs containing PRRS genes are identified, the porcine cells will be transfected with the unique restriction enzyme digested individual plasmid DNA(s) to isolate recombinant virus(s). The identity of the viruses will be confirmed as described above

d. Test immune response to vaccination and challenge studies.

Recombinant PAdV-3 expressing PRRS virus genes will be tested in 2-3 week old piglets. Animals will be immunized oronasally twice (four weeks apart) with recombinant PAdV-3 expressing Orf5, Orf3 or Orf5-Orf3. Control animals will be inoculated with PAdV-3. Animals will be monitored for the induction of mucosal and systemic PRRS virus specific immune responses using assays standardized in our laboratory. These animals will be challenged intranasally two weeks after second immunization with

virulent PRRS virus (obtained from ATCC). Animals will be observed for the development of clinical signs and virus shedding. Finally, animals will be euthanized and different tissues examined for gross and histopathological lesions. We predict that immunization of piglets with recombinant PAdV-3 expressing synthetic genes of PRRS virus will induce protective immune responses in pigs.

i) Indirect immunofluorescence assay: MARC- 145 cells were seeded in 96 well plate(s) and grown in DMEM (Dulbecco's minimum essential medium) overnight at 37°C with 5% CO₂. Next day, the cells were washed with PBS (phosphate buffered saline and incubated with 0.2 ml of media containing 600 TCID₅₀/ ml of PRRS virus. The cells were incubated for 2-3 days, when cells started showing cytopathic effects. The cells were fixed with 100% cold ethanol for 20 min. Finally, the plates were dried at room temperature and washed once with PBS. Test sera were diluted two fold serially ([0 day;1:2-1:128] [12 day: 1:2-1:1024] and 100µl of each sample was transferred to the infected cell plate. The plates were incubated for 45 min at 37°C. The plates were washed with PBS and incubated with rabbit anti-swine IgG conjugated to FITC (fluorescein isothiocyanate). After incubating for 45 min at 37°C, the plates were washed with PBS and examined under a fluorescent microscope. Each plate contained a positive control (PRRS antibody positive sera of known titer), a negative control (sera containing PRRS titer ≤ 1:2). In addition, uninfected cells were used as control also. The reciprocal of highest serum dilution showing fluorescence determined the titer of the sera.

ii) Virus neutralization test: Initially, 2 fold serial dilutions of heat inactivated sera were made in DMEM in 96 well flat bottom tissue culture plates. About 200 µl of each diluted sera and 200 ul of PRRS virus suspension (containing 200 TCID₅₀) were incubated for 1

h at 37 °C. The incubated mixtures were transferred to MARC-145 cells in triplicates in 96 well plates. The plates were incubated at 37°C with 5% CO₂. The cells were observed for the appearance of cytopathic effects for 5 days. The reciprocal of the highest dilution of sera which inhibited the development of cytopathic effects produced by PRRS virus in MARC-145 cells defined the neutralization titer of the sera.

c) *Western blot.* Monolayers of swine testicular (ST) cells (1X10⁵ per well) in 6 well tissue culture dishes were infected with recombinant PAdV-3 at a multiplicity of infection (MOI) of 1 and harvested at 24 h post infection. The cells were lysed in 100 µl of radioimmunoprecipitation buffer (RIPA) buffer (0.15M NaCl, 50 mM Tris-HCl, pH 8.0, 1% NP40, 1% deoxycholate, 0.1% SDS). Proteins from the lysates of PAdV-3 infected ST cells were separated on 10% sodium –dodecyl –sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) under reducing conditions, transferred to nitrocellulose membrane and blocked in tris-buffered saline (TBS) containing 3% skim milk. The membrane was probed with anti-PRRSV sera. The membrane was washed three times, exposed to goat anti swine IgG (H+L) (Jackson ImmnoResearch labs) conjugated to alkaline phosphatase, and developed using BCIP/NBT substrate (Sigma) according to manufacturer's directions.

d) *Virus isolation.* Pieces of lung tissue of individual pig were collected and placed into tubes containing 2.4 mm Zicornia beads and 1 ml of media. Tubes were vibrated on mini-bead beater to prepare lung homogenates. Homogenates from samples collected from the same pig were pooled together. A 10 fold serial dilution of each sample was made in media. Hundred µl of each sample dilution (in triplicate) was added to MARC-145 cells seeded in 96 well plates. The plates were incubated at 37°C in CO₂ for at least 5 days.

The cells were observed for the development of cytopathic effects. Appropriate positive and negative controls were included in each plate. The highest dilution showing cytopathic effects determined the titer of the virus.

d) Results:

A). Construction of synthetic PRRS genes

a) Sequence analysis of the PRRS genes Orf5, Orf3 and OrfM: The DNA sequences of three PRRS genes, open reading frame (Orf) 5, Orf3 and OrfM were retrieved from the 15 kb PRRS genome using the GenBank DNA database. The DNA sequences of Orf5, Orf3 and OrfM were adjusted to encode the same amino acid sequence using codons that are optimally used in pigs to ensure high levels of expression. Also, the DNA sequence was analyzed for the presence of cryptic RNA splice sites using the Splice Site Prediction software by Neural Network (Berkley Drosophila Genome Project). The RNA splice sites were removed from the sequences, while maintaining optimal codon usage (the optimal codon usage is based on the high frequency usage of a codon for an amino acid in highly expressed proteins in pigs [this is based on the analysis of thousands of proteins expressed in pigs, for which the amino acid sequences are available], this is verified by DNA sequencing) and the amino acid sequence. Within the Orf5, Orf3 and OrfM sequences, restriction enzyme sites were engineered into the DNA sequence without altering the amino acid sequence (verified by DNA sequencing).

b) Construction of synthetic Orf5, Orf3 and OrfM: Overlapping 100 base pair oligonucleotides were synthesized for construction of a 621 base pair Orf5 synthetic sequence *in vitro*. Eight oligonucleotides were designed in pairs with a 30 base pair overlap. Oligonucleotide pairs were annealed at 70°C for 30 min and the DNA sequence was extended using DNA polymerase (Klenow Fragment). The resulting 170 base pair double stranded DNA fragments were analyzed by Polyacrylamide Gel Electrophoresis (PAGE), isolated and then blunt-end ligated into the pBluescriptKSII⁺ vector at the *Sma*I site. The *E.coli* cells were transformed by plasmid DNA. In total, four Orf5 fragments (Fragment 5A, 5B, 5C and 5D) have been constructed and the cloning products have been analyzed by restriction digestion with Orf5 specific sites and DNA sequencing. Each fragment is flanked by corresponding restriction enzyme sites for ligation of the fragments to form a full-length Orf5 sequence. Fragment 5A, which represents the Orf5 N-terminus, is flanked with a *Bam*HI site at its 5' end and a *Kpn*I site at its 3' end. Fragment 5B has a corresponding *Kpn*I site at its 5' end for ligation with Fragment 5A, and a *Pst*I site at its 3' end for ligation with fragment 5C. Ligation of fragment 5D (Orf5 C-terminus) to 5C will utilize a *Stu*I restriction site present at the corresponding ends of each fragment. Construction of the full-length has been accomplished by sequential ligation of each fragment into the pBluescriptKSII⁺ containing fragment 5D (Orf5 C-terminus) followed by transformation of *E.coli* cells (Fig 1A). The identity of the synthetic gene was confirmed by the DNA sequence of the synthetic gene.

A 543 base pair synthetic Orf M was constructed in the same manner using four pairs of oligonucleotides for production of four double stranded DNA fragments (fragments M-A, M-B, M-C and M-D). Annealing of oligonucleotides, extension and

analysis of the double stranded DNA fragments were performed as described above.

Fragment M-A (OrfM N-terminus) was ligated into the pUC18 vector at the restriction sites *EcoRI* and *XbaI*. Ligation of fragment M-B with M-A has been performed at the *XbaI* site. The C-terminus of OrfM, fragment M-D, was ligated into pUC18 at the *BamHI* and *HindIII* restriction sites. Ligation of fragment M-C with M-D at the *BamHI* site has been performed. Finally, the M-C/M-D fragment and the M-A/M-B fragment were digested at an internal site with *MunI* followed by ligation and transformation of *E. coli* cells (Fig. 1B). The identity of the synthetic gene was confirmed by DNA sequencing of the synthetic gene.

The full-length synthetic Orf3 DNA sequence is 783 base pairs in length and was constructed in the same manner using five pairs of synthetic oligonucleotides. The oligonucleotides have been annealed and extended in double stranded DNA fragments as described above. The Orf3 fragments, designated 3-A, 3-B, 3-C, 3-D and 3-E have been analyzed and isolated by PAGE. All fragments have been subcloned into the pTZ18A vector by blunt-end ligation followed by transformation of *E. coli* cells. The ligation of the N-terminus of Orf3 (fragment 3-A) with fragment 3-B at a *SacI* site has been accomplished. This was followed by ligation of fragment 3-C onto fragment 2-B at a *BlnI* site. The C-terminus of Orf3, fragment 3-E, was subcloned into pTZ18A at the *BglII* and *HindIII* sites followed by ligation with fragment 3-D at a *BglII* restriction site. The full-length Orf3 synthetic sequence was constructed by ligation of fragment 3-A/3-B/3-C with 3-D/3-E at an internal *EaeI* site (Fig. 1C). The identity of the synthetic gene was confirmed by DNA sequence of the synthetic gene.

B) Construction of full length plasmids and recombinant viruses

a) Construction of plasmids

i) Construction of pPAV300CI. The transfer plasmid pPAV300CI was constructed by cloning a chimeric intron sequence into the *Sna*BI site of the E3 region in pPAV300⁵.

This intron was amplified from the pCI-Neo vector (Promega) by PCR using primers (5' GTAGTTAACGTAAGTATCAAGGTTACAAGAC 3') and (5' GTATACGTACTGTG GAGAGAAAGGCAAAG 3'). A 137 bp amplified product was isolated which has a *Hinc*II site at its 5' end and a *Sna*BI half site at its 3' end for reconstruction of the *Sna*BI site after blunt-end ligation in the appropriate orientation. The chimeric intron sequence consists of a human *beta-globulin* gene 5'-donor site taken from the first intron, together with the branch and 3'-acceptor site taken from the intron of an *immunoglobulin* gene heavy chain variable region⁸.

ii) Construction of recombinant PAV-3 plasmids containing PRRS virus Orf 5, 3 and

M.

A 609 bp Orf 5 sequence was isolated by digestion with *Bam*HI and *Hind*III from pGEM7zf (Fig. 2A). Orf 3 (831 bp) was isolated by digestion with *Eco*RI and *Hind*III (Fig. 2A). Digestion with *Sph*I and *Sac*I of pGEM7zf- containing Orf5-IRES (internal ribosomal entry site)-OrfM (Fig. 2B) resulted in an 1836 bp fragment. These sequences were blunt-end repaired by Klenow polymerase and ligated into pPAV300CI at the *Sna*BI restriction site, downstream of the chimeric intron to avoid the occurrence of aberrant mRNA splicing products from cryptic 5'-donor splice sites within the PRRS sequences. The *Sph*I-*Spe*I fragments of the pPAV300CI containing the individual PRRS transgene insertions in the E3 region were isolated and individually recombined with *Sna*BI

linearized pFPAV300⁵ plasmid in BJ 5183 *Escherichia coli* cells⁷. Full-length clones were rescued for E3 insertion of synthetic PRRS genes Orf3 (pFPAV310a), Orf5 (pFPAV312a) and Orf5-IRES-OrfM (pFPAV317a) (Fig. 3).

b) Construction of Recombinant PAdV-3 expressing PRRS virus Genes

To increase the expression of PRRS virus genes, we initially inserted a chimeric intron sequence into the E3 region of plasmid pPAV300 creating plasmid pPAV300CI. The full-length synthetic Orf3 or Orf5 were individually inserted into the E3 region of pPAV300CI and finally recombined into the E3 region of the pFPAV300 genome in the same transcriptional orientation as E3, using the homologous recombination machinery of *E. coli* BJ5183⁷. The PacI-digested pFPAV310a or pFPAV312a plasmid DNA was transfected into ST / VIDO RI cells (Fig. 3). The cells showing 50% cytopathic effects were collected, freeze-thawed three times and recombinant viruses were propagated in ST cells. The recombinant viruses were named pAV310a (pFPAV310a) and PAV312a (pFPAV312a). The viral DNA was extracted and analyzed by agarose gel electrophoresis after digestion with restriction enzyme. Expected bands were observed in PAV300 and, recombinant viruses PAV310a and PAV312a. Two assays were used to detect the expression of recombinant proteins. First, the expression of protein was detected by IIF. As seen in fig. 4, anti-PRRS serum detected recombinant proteins in the cytoplasm of PAV310a (panel D) or PAV312a (Panel B) infected cells but not in PAV300 (Panel A, C) infected cells. Secondly, the expression of recombinant protein was analyzed by Western blot. As seen in Fig. 5, anti-PRRS serum detects a specific protein in PAV312a infected cells but not in PAV300

infected cells. Similarly, anti-PRRS serum detects a specific protein in PAV310a infected recombinant Orf5 are expressed in cells infected with PAV310a and PAV312a, respectively.

In addition to the proposed objectives, we also constructed the full-length plasmid pFPAV317a containing Orf5-IRES-OrfM inserted in the E3 region of PAV300. The PacI-digested plasmid pFPAV317a DNA was transfected into ST cells or VIDO R1 cells. Repeated transfections of cells with plasmid pFPAV317a DNA did not produce any cytopathic effects.

C) Testing immune response to vaccination

The recombinant viruses were passaged and grown in VIDO R1 cells to make viral stocks. In order to determine the immune response, 2-3 week old crossbred piglets were randomly allocated in different groups. All pigs were seronegative for PRRS antibodies as tested by ELISA (Herdcheck PRRS 2XR kit). However, all pigs had low level of anti-PAV-3 titers in the serum. The piglets (10 per group) were vaccinated twice, three weeks apart with recombinant PAV310 (expressing wild-type Orf3), PAV312 (expressing wild- type Orf5) PAV310a (synthetic Orf3), PAV312a (synthetic Orf5), or PAV300 (E3 deleted; control) oronasally (5×10^7 IU/ml). At day 32, all pigs were challenged intranasally with 10^6 TCID₅₀/ml of PRRS virus (ATCC VR-2332 grown in pig lung). Animals were observed for the development of any clinical sign(s) and temperature. Blood for Serum was collected at days 0, 32, and 44, and analysed for humoral immune responses and virus isolation. In addition, blood was also collected in on day 32 and 44 days for isolation of PBMCs.

After two immunizations with PAV312, PAV312a, or PAV300, very low levels of antibody titers were detectable by indirect immunofluorescence (IIF) assay (Fig.6) at 0 day post challenge. There was no significant difference between vaccinated animals and control animals ($P>0.05$). However, 12 days post challenge, pigs immunized with either PAV312 or PAV312a developed higher antibody titers compared to pigs immunized with PAV300 as detected by IIF (Fig. 6). However, the difference was significant only in pigs immunized with PAV312a compared to pigs immunized with PAV300 ($P<0.001$) or PAV312 ($P<0.001$). Although low levels of PRRS virus neutralization (VN) antibodies (Fig. 7) were detected in pigs immunized twice with either PAV312 or PAV312a at 0 day post challenge, there was significant difference between pigs immunized with PAV300 to pigs immunized with PAV312a ($P<0.001$). However 12 days after challenge, pigs immunized with PAV312a developed significantly higher VN titer than pigs immunized with either PAV312 ($P<0.001$), or PAV300 ($P<0.05$) (Fig. 7).

Similarly, very low level of antibody titers were detected in pigs immunized with PAV310a or PAV300 as detected by IIF (Fig. 6) at day 0 post challenge. The level of antibodies increased in pigs immunized with PAV310a compared to PAV310 on day 12 post challenge as detected by IIF (Fig. 6). However, the difference was significant in pigs immunized with PAV310a than pigs immunized with PAV300 ($P<0.05$). Low levels of PRRS VN antibodies (Fig. 7) were detected in pigs immunized twice with PAV310 and PAV310a at day 0. However 12 days after challenge pigs immunized with PAV310a developed significantly higher VN titers than pigs immunized with either PAV310 ($P<0.001$) or PAV300 ($P<0.05$)

To assess PRRSV-specific cellular immune responses, PBMCs were isolated from blood samples on day 32 and 44. PBMCs were seeded into 96 well plates (and stimulated with inactivated PRRSV virus (10µg/ml) or ConA (5µg/ml). At 48 h post stimulation, cell culture media were harvested and assayed using BIOSOURCE swine INF-γ. Although INF-γ could be detected in ConA stimulated cells, none of the samples stimulated with inactivated PRRSV was positive for INF-γ.

To determine the virus load, 10 fold serial dilution of homogenates of pieces of lung from each pig were added to the monolayer of MARC-145 cells. The cells were observed for the development of cytopathic effects. There was really no significant difference in the PRRS virus load between animals immunized with PAV300, PAV310, PAV310a and PAV312 (titers ranged from 10^4 - 10^5 /pig) at 12 days post challenge (Fig.8). However, pigs immunized with PAV312a contained significantly less virus than pigs immunized with PAV300 ($P < 0.001$) or PAV310 ($P < 0.001$). Although there is significant decrease, there is still enough virus which can lead to development of disease once the immune response subsides.

No significant difference in clinical signs including rectal temperature could be observed amongst the groups. Moreover, no significant gross lesion(s) could be detected in the lungs resulting from the virus challenge in any of the groups.

e) Conclusions & Recommendations

One of the major impediments to effective vaccines is delivery. We have chosen to develop PAdV-3 as a live viral vector for delivery of vaccine antigens. Initially, we developed a simple and efficient way of constructing recombinant PAdV-3 (using

homologous recombination machinery of *E. coli*), identified sites for insertion of foreign genes, and demonstrated the ability of PAdV-3 to express vaccine antigens.

Earlier, we also showed that recombinant PAdV-3 expressed low levels of PRRS glycoprotein (wild-type) vaccine antigens. Moreover, immunization of pigs with recombinant PAdV-3 expressing PRRS wild-type glycoproteins produced did not induce protection in the animals from PRRS challenge. Here we demonstrated that use of synthetic (codon optimized) PRRS virus glycoprotein genes helped to increase the level of expression of these glycoproteins. However, the level of vaccine antigen expression was not sufficient to induce protection in challenged pigs.

An ideal vaccine for PRRS should induce both humoral and cellular immune responses against the virus. So far, the best protection in pigs against PRRS disease has been achieved mainly by using live attenuated PRRS virus based vaccines. However, use of live attenuated PRRS virus vaccines have its disadvantages (as discussed in introduction). As such a delivery system is required which can efficiently deliver potential PRRS virus vaccine antigen(s) to appropriate cells/tissues of the pig for inducing protective immune responses. Although a number of strategies including DNA vaccination⁹ and vectored (e.g Bacille Calmette-Guerin vector¹⁰, herpes virus vector¹¹, Vaccinia virus¹²) vaccination are being evaluated for producing / delivering vaccines against PRRS disease in pigs, none of these strategies have so far been successful in inducing protection against PRRS disease in pigs for a number of reasons:

i) Choice of vaccine antigens: It is clear from different studies including our's that only Orf5 or Orf3 are not sufficient in inducing protective immune responses in pigs.

Studies have shown that Orf M is required for proper folding of Orf5, a requirement for inducing potent neutralizing antibody responses. Hence, other proteins encoded by PRRS virus have to be evaluated before finalizing these potential vaccine candidates.

ii) Inclusion of proteins inducing T cell responses: As both humoral and cellular immune responses are required for inducing protection against PRRS disease in pigs, the future recombinant vaccines should contain antigens, which induce a potential cellular immune responses.

iii) Choice of vaccine antigen delivery system: It is widely accepted that next generation of PRRS vaccine has to be produced by recombinant DNA technology. Since the best option to producing/delivering a cost effective PRRS vaccine is by using a vector system, we believe that PAdV-3 is an ideal vehicle for producing such vaccines. However, more studies need to be done (e.g. effect of insertion site, effect of different transcriptional elements, effect of targeting vector to antigen presenting cells to determine the full potential of this approach for delivering vaccines in porcine.

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g) Literature cited

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h) Appendices

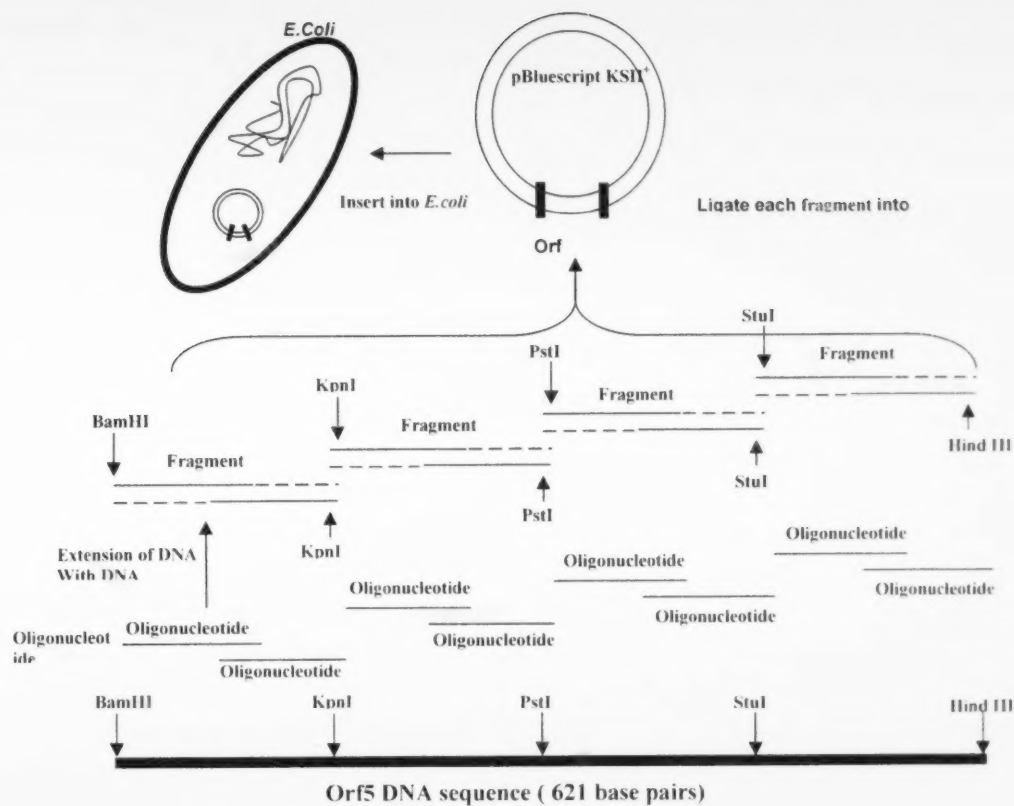


Fig. 1A. Schematic diagram showing the construction of synthetic Orf 5. Synthetic oligonucleotide DNA (—); Klenow DNA (-----); complete Orf (■). Restriction sites (BamHI, KpnI, PstI, StuI, HindIII) used to construct the full length Orf5

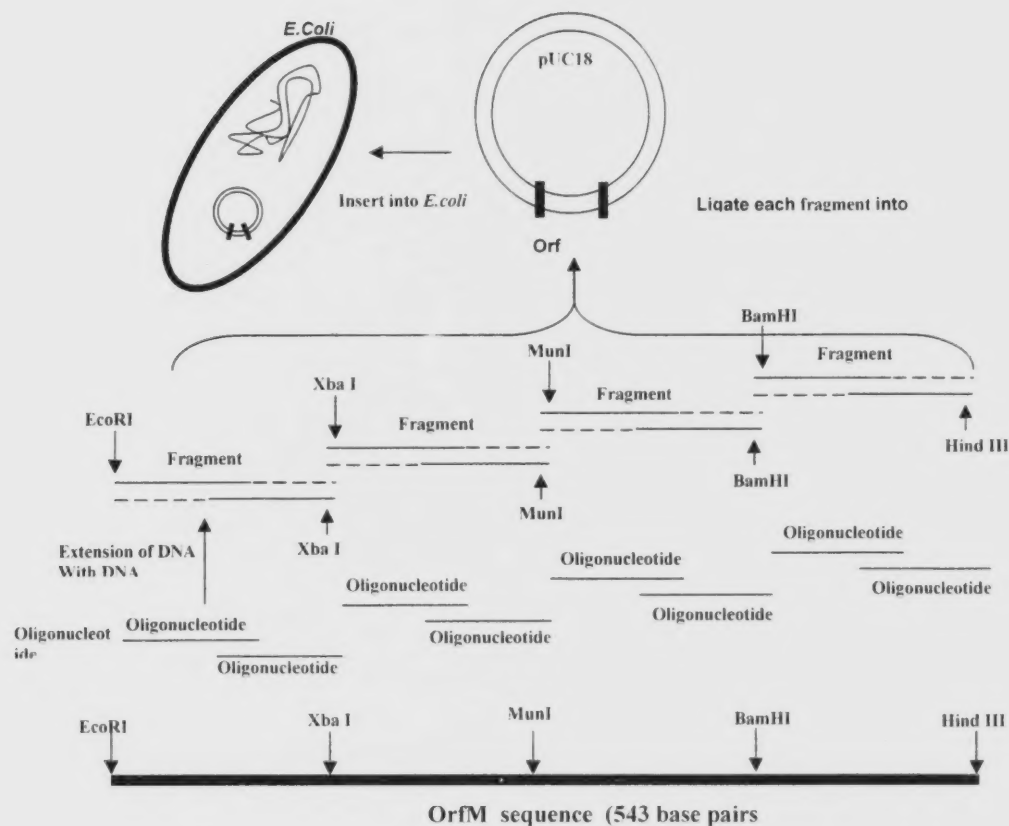


Fig. 1B. Schematic diagram showing the construction of synthetic Orf M. Synthetic oligonucleotide DNA (—); Klenowed DNA (-----). Complete ORF (■). Restriction sites (EcoRI, XbaI, MunI, BamHI, HindIII) used to construct the full length OrfM.

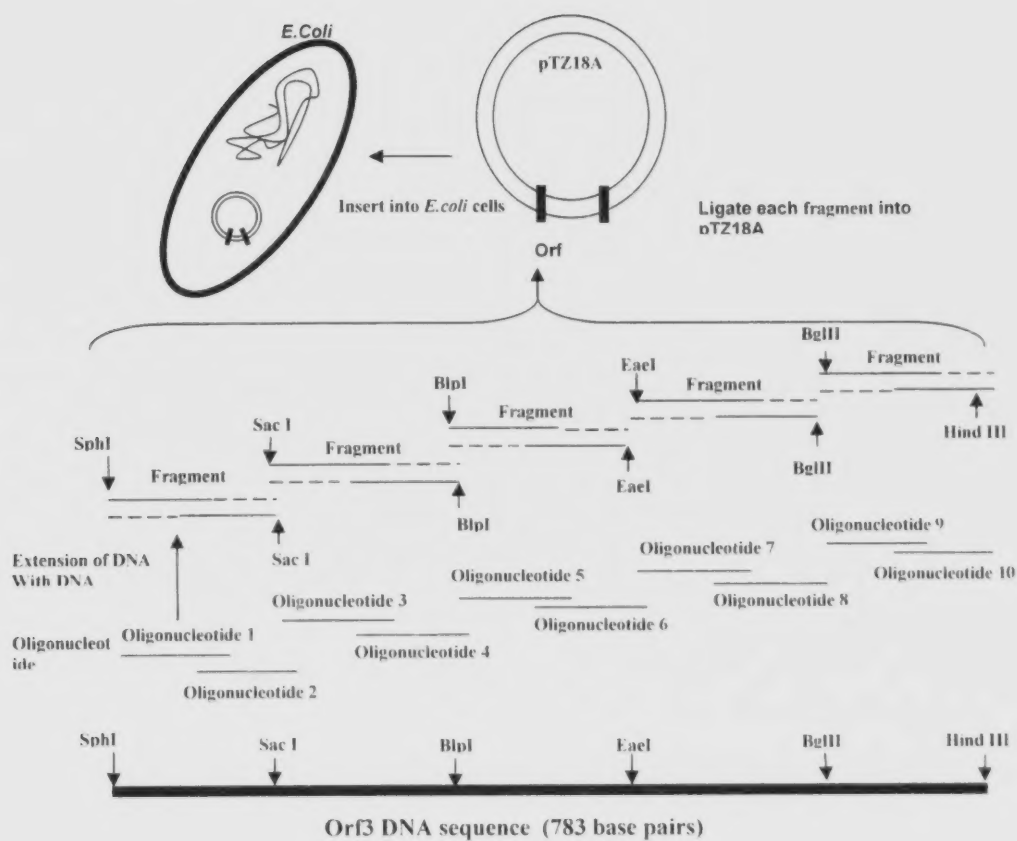


Fig. 1C. Schematic diagram showing the construction of synthetic Orf 3. Synthetic oligonucleotide DNA (—); Klenowed DNA (-----). Complete ORF (■). Restriction sites (EcoRI, XbaI, MunI, BamHI, HindIII) used to construct the full length Orf3.

A

Full-length PRRS Orf 5



Full-length PRRS Orf 3



B

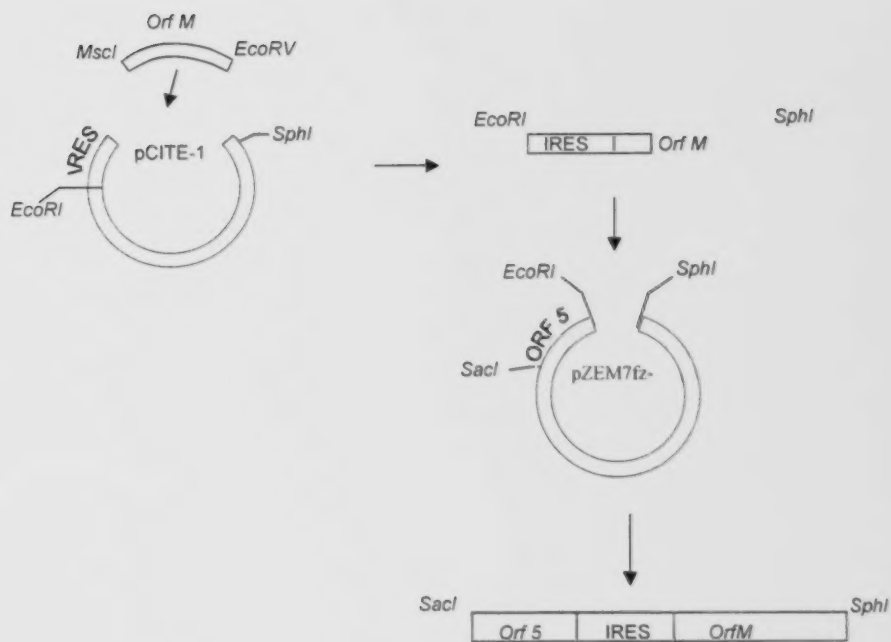


Fig. 2 Schematic diagram showing isolation of A) Orf5, Orf3; and B) Orf-IRES-Orf3 gene by digestion with restriction enzymes for cloning puposes.

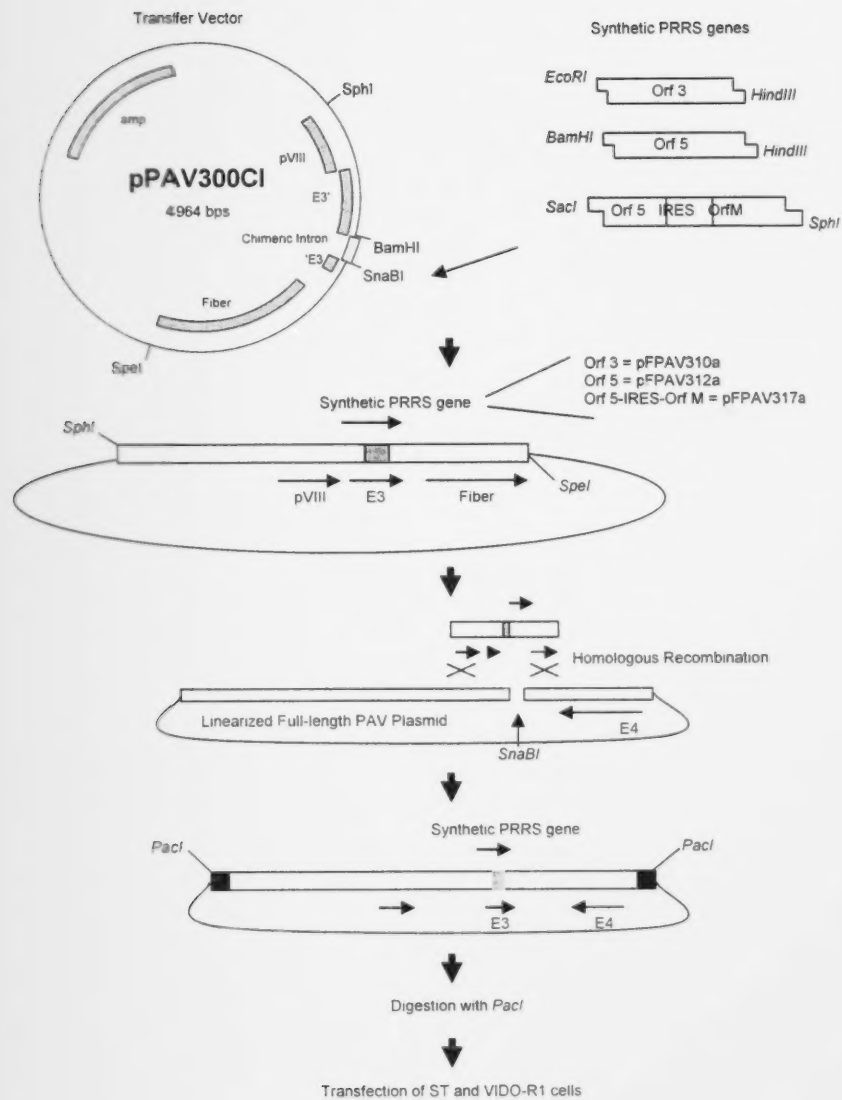


Fig. 3 Schematic diagram showing construction of recombinant PAdV-3

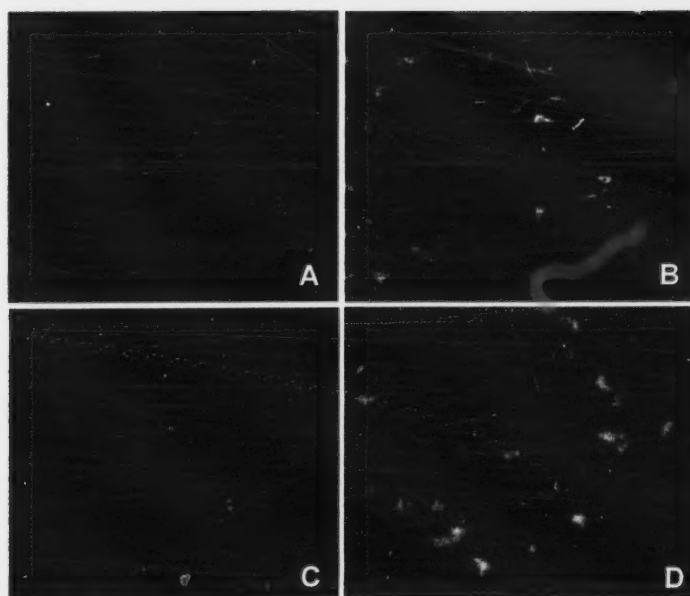


Fig. 4

Swine testicular cells in chamber slides were infected with PAV300 (panels A,C) , PAV312a (Panel B) or PAV310a (Panel D). After 203 days post infection, the cells were fixed with 100% cold ethanol for 20 min. The cells were incubated with 1:100 dilution of anti -PRRS virus serum for 45 min at 37⁰C. The cells were washed with PBS and incubated with rabbit anti-swine IgG conjugated to FITC (fluorescein isothiocyanate). After incubating for 45 min at 37⁰C, the plates were washed with PBS and examined under a fluorescent microscope (as described in the text)

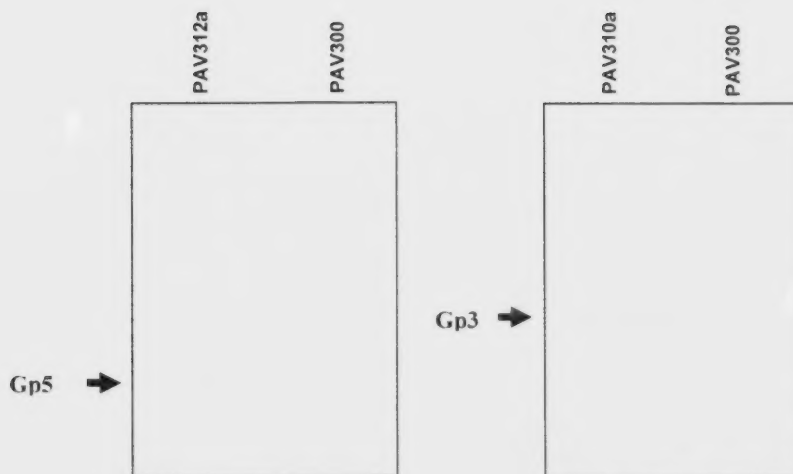


Fig. 5 Proteins from lysates of cells infected with PAV300, PAV310a or PAV312a were separated on 10% sodium dodecyl sulphate (SDS)-polyacrylamide gels (PAGE) and transferred to nitrocellulose membranes. The separated proteins were probed in Western blot using anti-PRRS virus serum (as described in the text).

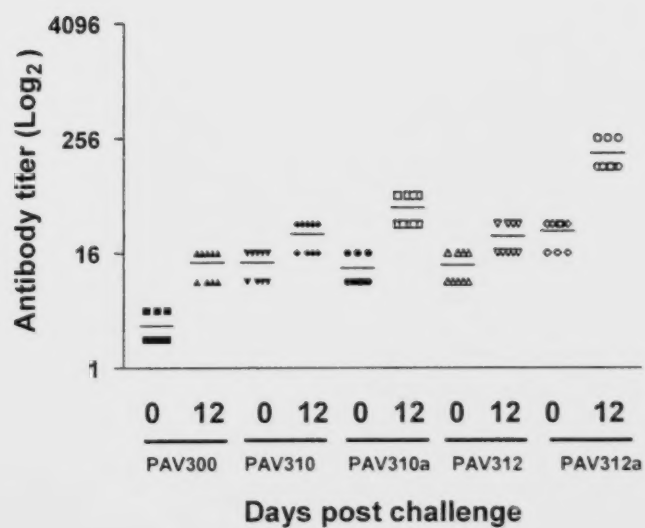


Fig 6. Antibody titers in sera as determined by IIF (Method described in the text).

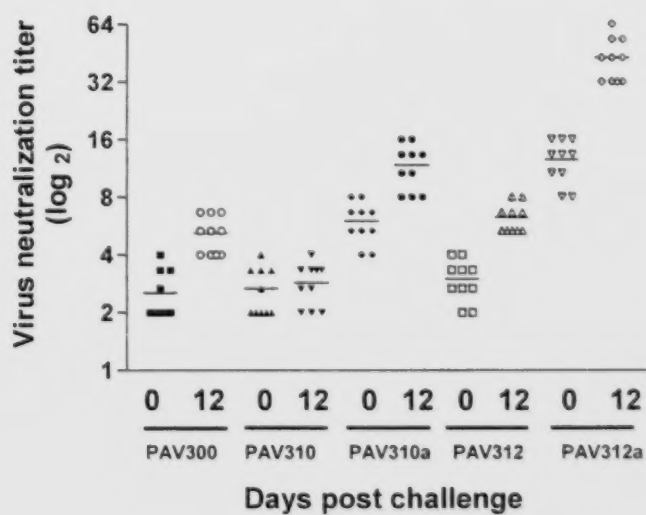


Fig. 7. Virus neutralization titers in sera of pigs. (Method described in the text)

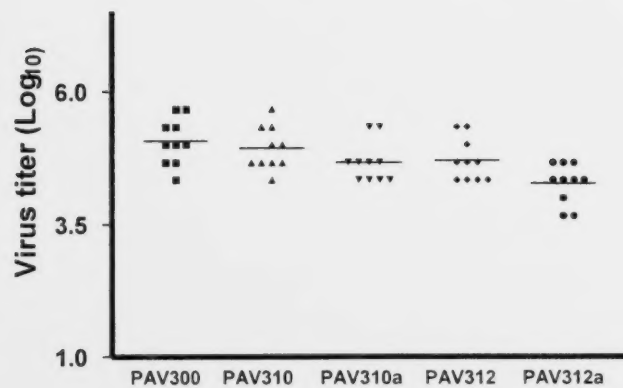


Fig. 8 Virus titers in lungs of infected pigs. (Method described in the text)

i) Administrative and other aspects:

a) PERSONNEL

Dr. Suresh Tikoo	Senior Research Scientist
Dr. G. Mutwiri	Senior Research Scientist
Dr. Zenip Akin	PDF
Ms. Satender	Technician

b) Publications: So far we have not published any papers. We are in the process of writing the publications.

c) Expense statement: A copy is attached

